View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by AJOL - African Journals Online

African Journal of Environmental Science and Technology Vol. 6(2), pp. 104-114, February 2012
Available online at http://www.academicjournals.org/AJEST
DOI: 10.5897/AJEST11.067
ISSN 1996-0786 ©2012 Academic Journals

Full Length Research Paper

Characterization of methomyl and carbofuran degrading-bacteria from soils of horticultural farms in Rift Valley and Central Kenya

Kevin Mbogo Omolo[1]*, Gabriel Magoma[1], Kamau Ngamau[2] and Tsanuo Muniru[3]

[1]Biochemistry Department, Jomo Kenyatta University of Agriculture and Technology, P. O. Box 62000-00200, Nairobi, Kenya.
[2]Horticulture Department, Jomo Kenyatta University of Agriculture and Technology P. O. Box 62000-00200, Nairobi, Kenya.
[3]Department of Pure and Applied Sciences, Pwani University College, P. O. Box 175 Kilifi, Kenya.

Accepted 30 May, 2011

The use of pesticides is very critical in protecting the farmers' investment in seeds, fertilizer and labour since they provide a sure cover from damage by pests. The use of pesticides is therefore inevitable and the environmental pollution due to pesticides and their residues will continue to be a challenge. In this study, bacterial strains capable of degrading methomyl (S-methyl-N-[(methylcarbamoyl) oxy]-thioacetimidate) and carbofuran (2, 3-dihydro-2, 2-dimethyl-7-benzofuranyl methylcarbamate) were isolated from soils sampled from horticultural farms with a history of pesticide usage. High pressure liquid chromatography was used to monitor biodegradation of both methomyl and carbofuran using reference standards and acetonitrile and water as mobile phases. Partial 16S rDNA sequence analysis indicated that the carbofuran-degrading strains were closely related to members of the genus *Pseudomonas* and *Alcaligenes* while the methomyl degrading strains were closely related to members of the genus *Flavobacterium* and *Alcaligenes.* The morphological and biochemical characteristics of the isolates also confirmed the phylogenetic signature. The study established that the activities of the esterase and phosphatase enzymes correlated well with biodegradative capability and recommends possible application of the isolates in the *in vivo* bioremediation of pesticide contaminated soils.

Key words: Pesticides, carbofuran, methomyl, biodegradation, bacteria.

INTRODUCTION

Xenobiotic compounds are widely distributed in the environment as a result of their widespread use as pesticides, solvents, fire retardants, pharmaceuticals, and lubricants. Several of these chemicals cause considerable environmental pollution and human health problems due to their persistence and toxicity (Rossberg et al., 1986). Agricultural modernization due to increased market demand for agricultural products has greatly facilitated the industrial production and use of pesticides for pest management and vector control thus the diverse environmental contamination with pesticides (Ngowi et al., 2007). Carbamates, synthetic organic chemicals, are highly poisonous pesticides that have found wide usage in agricultural farms as insecticides, fungicides, herbicides, nematicide and acaricides (WHO, 1986). The non-target toxicity of carbamates extends from human beings to both aquatic and terrestrial organisms with high sensitivity in fish and earthworms (WHO, 1986). Some of the carbamates used in horticultural farming include carbofuran (in form of Furadan), carbaryl, aldicarb and methomyl. Many researchers have reported the biodegradation of various pesticides under different physiological conditions and isolated many bacterial

*Corresponding author. E-mail: omolokevin@yahoo.com. Tel: +254-723-623-791.

species. It has thus become increasingly possible to isolate microorganisms that are capable of degrading xenobiotic and recalcitrant compounds from environments polluted with toxic chemicals (Gibson and Harwood, 2002). This study focused on the isolation and characterization of methomyl and carbofuran degrading bacterial strains in selected agricultural soils.

MATERIALS AND METHODS

Sampling

Soil samples were collected from horticultural farms in three geographically distinct regions; Naivasha which is in Northwest of Nairobi located at 0° 43´ 0˝ South and 36° 26´ 0 ˝East and Gilgil which is located in the following geographical co-ordinates 0° 13´ 0˝ South, 36° 16´ 0˝ East in Rift Valley and Thika 1° 3´ 0˝ South 37° 5´ 0˝ East in Central Kenya. Stratified random sampling method was used to collect soils with the geographical regions forming three strata. From each region, plots (farms) were identified by simple randomization. Cross-sectional sampling was done on the upper (5 to 20 cm deep) soil from five locations within a given plot of ~100 m^2. Samples were transferred in glass jars and stored at 25°C until used. Aerobic conditions for sampling, medium preparation and culture handling were maintained throughout the subsequent enrichment process.

Media composition and culturing

The organisms were cultivated in phosphate buffered mineral salts medium (MSM) as described by Chaundry and Ali (1988). The MSM contained the following constituents, in grams per liter: K_2HPO_4, 4.8; KH_2PO_4, 1.2; NH_4NO_3, 1.0; $MgSO_4$ $7H_2O$, 0.2; Ca $(NO_3)_2$.$4H_20$, 0.4, and Fe_2 $(SO_4)_3$, 0.001. The media was supplemented with carbofuran or methomyl as the sole carbon source to a final concentration of 2 mM. All the solutions, cultures and media were prepared and maintained using aerobic techniques which included covering media with cotton wool and shaking to allow air circulation in the cultures. Cultures were incubated on a shaker (100 rpm) at 30°C and monitored for the loss of carbofuran or methomyl using high pressure liquid chromatography while turbidity increase due to bacterial growth was monitored using spectrophotometer (Shimadzu UV240) at OD_{600}.

Culture enrichment and isolation

The isolation of methomyl- and carbofuran-degrading bacteria was done using a modified enrichment culture technique described by Chaundry and Ali (1988). Approximately 1 g of each soil sample was suspended in 10 ml of mineral medium (MSM) containing 2 mM of carbofuran or methomyl as the sole carbon source. Cultures were grown in 100 ml- culture flasks under aseptic conditions at 30°C with shaking in a rotary shaker at 100 rpm for 18 days. Using wire loop, cultures were then streaked into agar plates containing mineral salt medium supplemented with 2 mM of methomyl or carbofuran. Single colonies obtained were re-suspended in basal medium (MSM) containing 2 mM of methomyl or carbofuran for 14 days to confirm the ability of the isolates to utilize methomyl or carbofuran. The cultures were then streaked into plates containing Luria-Bertoli medium (LB) with the following components, in grams per liter: tryptone, 10; yeast extract, 5; NaCl, 5 and mineral agar 15. Sub-culturing was done periodically on pesticide-supplemented medium (agar) until pure colonies were obtained. All solid media contained 0.1 g/L cycloheximide (Sigma-Aldrich, Steinheim, Germany) to suppress fungal growth (Maarten et al., 2007).

Morphological and biochemical characterization of the isolate

Cell morphology was determined by a phase contrast Zeiss Axiophot microscope (Carl Zeiss, Oberkochem, Germany) supplemented by the classical gram staining method (Bartholomew, 1962). Cell motility was assesed by direct microscopic observation from cultures in the growth phase (in SIM agar; pH 7.3). Biochemical tests for nitrate reduction, indole production, citrate utillization, hydrogen sulphide production and acid production were done according to the procedures described by Cappuccino and Sherman (2002).

Assay of phosphatase activity

The isolates were first grown in a low phosphate media suitable for formation of both repressible and constitutive alkaline phosphatase from bacteria. The composition of the media included; peptone water 3 g/L, yeast extract 1 g/L, NaCl 31.2 g/L, $MgCl:6H_2O$ 5.39g/L, $Fe(NH4)_2SO_4$, Trizima base 12.1 g/L, pH 7.4 and each isolate was cultured in 10 ml of the medium in universal bottles with reciprocal shaking at 27°C for 24 h.

Phosphatase activity was estimated in duplicate samples by monitoring the liberation of p-nitrophenol from p-nitrophenyl phosphate (disodium salt; BDH) using a modified procedure described by Bolton and Dean (1972). One milliliter of cultured medium was incubated with 1 ml of 50 mM p-nitrophenol phosphate solution, 1 ml of 0.5 M Tris-HCl buffer pH 8.2 and 1 ml of mineral salts; 0.4 M NaCl, 0.05 M $MgCl_2$, 0.01 M KCl and 0.01 M $CaCl_2$. The mixture was incubated for 60 min at 28°C before addition of 4 ml of 0.2 M sodium hydroxide. The p-nitrophenolate liberated was measured using spectrophotometer at 405 nm verses p-nitrophenol standard.

Assay of esterase activity

Esterase activity was assayed using Tween 80 as described by Sierra (1957). The media contained (g L^{-1}): peptone 10.0, NaCl 5.0, $CaCl_2.2H_2O$ 0.1 and agar 18.0, pH 7.4. To the sterilized culture media, previously sterilized, Tween 80 was added at a concentration of 1% (v/v). The medium was inoculated with the isolates and the presence of halos was observed and the halo diameters recorded. The enzymatic index was then calculated by dividing the halo diameter by colony diameter.

Chromatographic analysis of carbofuran and methomyl degradation

The decline in carbofuran and methomyl concentration upon degradation was monitored using high pressure liquid chromatography (HPLC) (VP shimadzu) on a reverse phase C-18 column, 25 cm × 4.6 mm, fitted with a C-18 silica reverse phase guard column and equipped with a UV detector and retention time identified using reference standards at 254n m, 70% acetonitrile and 30% water for carbofuran and a wavelength of 235 nm, 30% acetonitrile and 70% water for methomyl (Figures 1 and 2, respectively). The HPLC pumps were set at a flow rate of 1ml/min at a temperature of 27°C.

The mobile phase was prepared by first washing the 1000 ml-bottles and oven drying for an hour before filling with 800 ml of either acetonirile (HPLC grade) or double distilled water. The water or acetonitrile was then sonicated and de-gassed for 1 h before connecting to the pumps (LC-10AT VP-Shimadzu A and B).

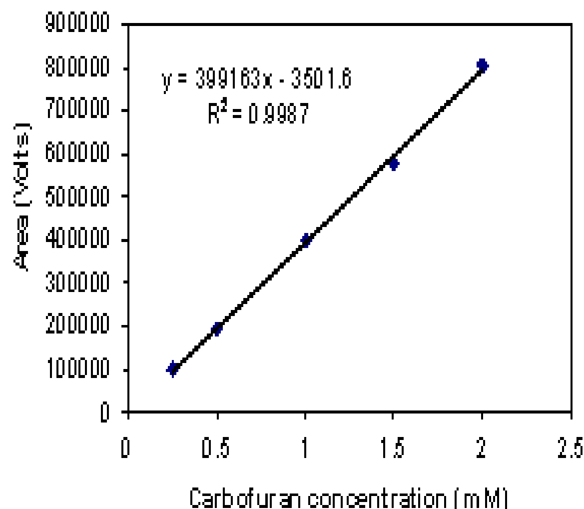


Figure 1. Standard curve for carbofuran.

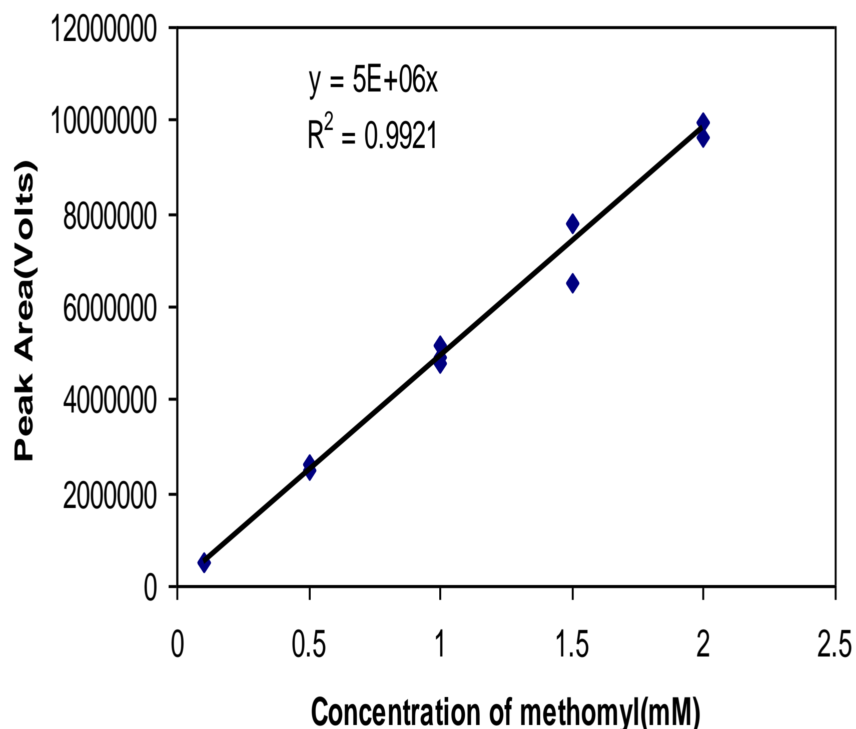


Figure 2. Standard curve for methomyl.

Molecular characterization

The isolates were further subjected to molecular characterization by extracting genomic DNA from the isolates using a standard protocol (Sambrook and Russell, 2001). The DNA was then subjected to polymerase chain reaction (PCR) amplification and partial sequence analysis of the 16S rDNA gene. Nearly full-length 16S rRNA gene sequences were PCR-amplified using bacterial primer pair 8F forward 5'-AG (A/G) GTTTGATCCTGGCT-3') and 1492R reverse, 5'-CGGCTACCTTGTTACGACTT-3' (Sigma) according to the position in relation to *Escherichia coli* gene sequence (Embley and Stackebrandt, 1994). Amplification was performed using Model PTC-100 thermal cycler (MJ research inc., USA). The amplification mixture comprised of 1.25 U of genescript Taq, 1.0 µl (5-pmol) of 8F forward primer, 1.0 µl (5-pmol) of 1492R reverse primer, 1 µl of template DNA, 2.5 µl of dNTPs mix (2.5 mM), 4.0 PCR 10x buffer (genescript) and 30.25 µl of PCR water while the control contained all the above except the DNA template. Reaction mixtures were subjected to 35 cycles: Initial denaturation of the DNA at 94°C for 5 min, denaturation at 94°C for 45 s, primer annealing at 52°C for 30

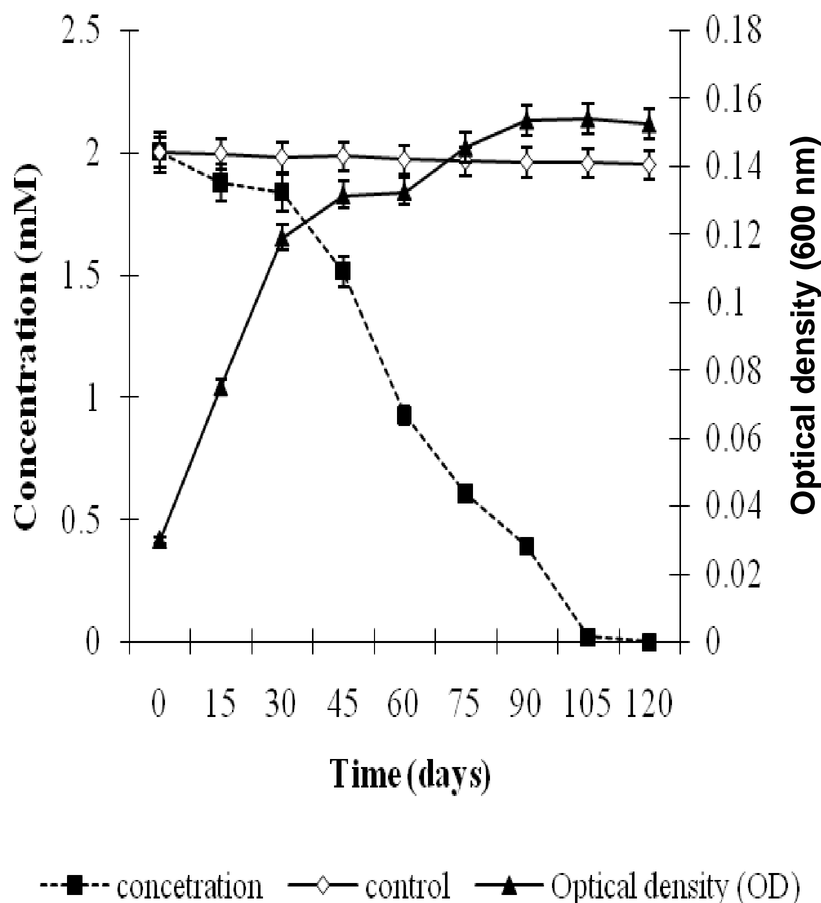


Figure 3. Growth of isolate C-9 on carbofuran. Growth was performed aerobically in 2 mM carbofuran at 30°C. The control was exposed to same conditions as test samples but lacked innoculum.

s, chain extension at 72°C for 1.5 min and a final extension at 72°C for 5 min (Roux, 1995). Amplification products (7.0 µl) were separated on a 1% agarose gel in 1X TAE buffer and visualized by ethidium bromide staining (Sambrook et al., 1989). The PCR products were purified using the QIAquick PCR purification Kit protocol (Qiagen, Germany) according to manufacturer's instructions and then resolved by electrophoresis on 1% agarose gel. Sequencing of purified PCR products was done without cloning, using a commercial service provider. Alignments were checked and corrected manually where necessary, based on conserved regions using Chromas pro and Mega 4 programs. The 16S rRNA gene sequences were compared to sequences in the public database using Basic Local Alignment Search Tool (BLAST) on the National Center for Biotechnology Information (NCBI) website (http://www.ncbi.nih.gov) in order to determine similarity to sequences in the Genebank database (Shayne et al., 2003). The 16S rDNA gene sequences with high similarities to those determined in the study were retrieved and added to the alignment based on BLAST results. Phylogenetic trees were constructed by maximum likelihood method.

Nucleotide sequence accession number

The 16S rRNA gene sequence of the methomyl and carbofuran-degrading isolates, M-14, M-23, C4 and C-9, were deposited in GenBank under accession numbers D14019, GQ375790, GQ375790 and EU930874, respectively.

RESULTS AND DISCUSSION

Enrichment culturing and isolation of methomyl and carbofuran degrading bacterial strains

Using independent enrichment steps with carbofuran (2 mM) or methomyl (2 mM) as the sole carbon and energy source, four bacterial strains were isolated from soils sampled from selected horticultural farms in Rift Valley and Central Kenya. The isolated strains were found to be rod shaped and gram negative bacteria. Two of the isolates (C-4 and C-9) were found to degrade carbofuran completely within 100 days and the other two (M-23 and M-14) were found to degrade methomyl completely within 40 days as compared to the control in which there was no significant decline in concentration. As expected, the decline in bacterial growth was observed in all isolated strains as the concentration of pesticides approached

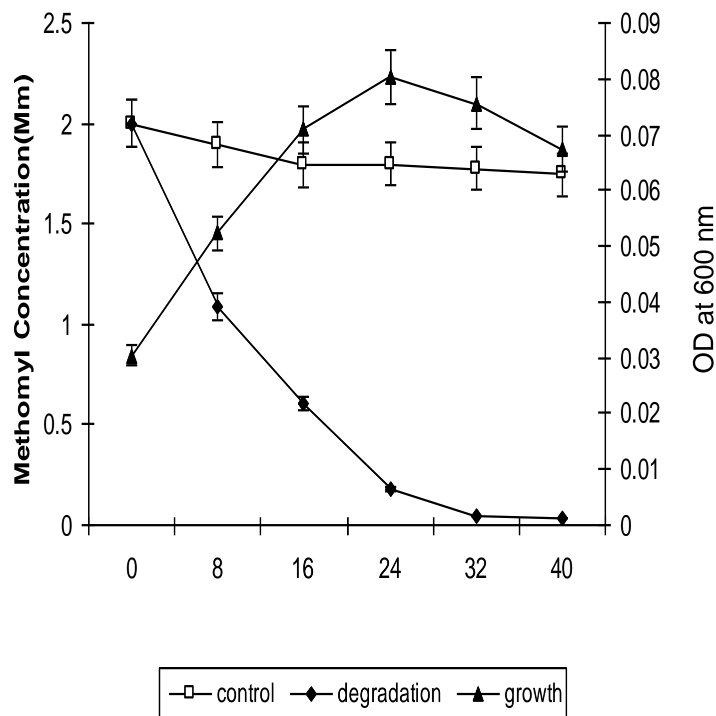


Figure 4. Growth of isolate M-23 on methomyl. Growth was performed aerobically in 2 mM methomyl at 30°C.

depletion (Figures 3 and 4). However, for carbofuran degrading isolates, the decline in cell biomass was not sharp and may be attributed to the utilisation of the metabolite by the strains as observed by the decline in the concentration of major carbofuran metabolites (Figure 5).

Carbofuran was detected at a retention time of 4.1 min at a wavelength of 254 nm using acetonitrile to water in ratio of 7:3. Three unidentified metabolites were observed at retention times 1.9. 2.0 and 2.3 min, respectively and as expected, their concentration increased steadily with the decrease in the concentration of carbofuran (Figure 5). Total depletion of the carbofuran was observed within 100 days and this was followed by the decline in the concentration of two of the metabolites with retention times 1.7 and 2.0 min. One new peak was again observed with the depletion of the two metabolites at a retention time of 3.6 min. The decline in the concentration of carbofuran was monitored for over 100 days with the metabolites observed within the first 20 days of incubation (30°C) for both the isolated strains. The degradative pathways of the two strains (C-4 and C-9) on carbofuran seemed identical as similar chromatographic profiles were observed with both strains. Various pathways have been proposed for carbofuran mineralization by microorganisms with oxidation and hydrolysis being the dominant pathways. This study did not establish degradation pathway for both methomyl and carbofuran, however, the major carbofuran biodegradation metabolites that have been reported includes carbofuran phenol, 3- ketocarbofuran, and 3-Hyroxycarbofuran (Seo et al., 2007). Chaudhry (2002) also reported microbial metabolites of carbofuran which include 2, 3-dihydro-2, 2-dimethyl-7-hydroxybenzofuran, 3-hydroxycarbofuran, 3-hydroxycarbofuran-7-phenol,3-ketocarbofuran and 3-ketocarbofuran-7-phenol.This study did not establish the metabolites observed, however, the decline in the concentration of the metabolites may have been due to their utilization by the strain after the depletion of the carbofuran. This argument may be supported by the fact that the cell biomass of the strains never decreased sharply with the depletion of the carbofuran. However, the preference of carbofuran to the metabolites as sole carbon source may be noted by the decrease in the cell biomass after carbofuran depletion.

Chromatographic profiles for methomyl degradation

Methomyl was detected at a retention time of 4.9 min at a wavelength of 235 nm with water to acetonitrile ratio of 7:3 (Figure 6). One unidentified methomyl metabolite was detected at a retention time of 4.1 min within five days of incubation. The metabolite later disappeared and could not be detected after 28 days of incubation at 30°C. Upon depletion of the first metabolite (Figure 6), two other metabolites were detected at retention times of 1.4 and 6.1 min which eventually disappeared in the next 10 days

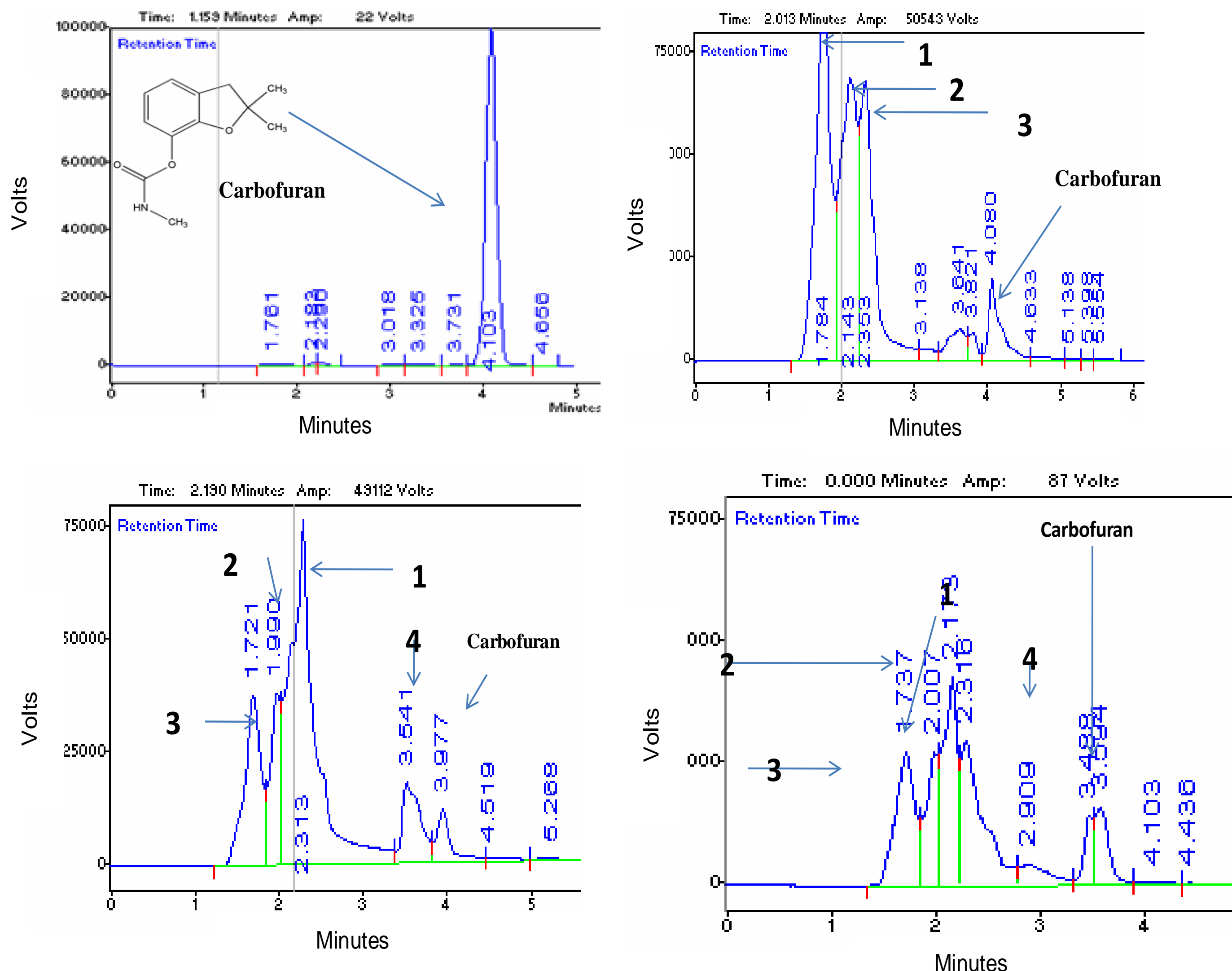


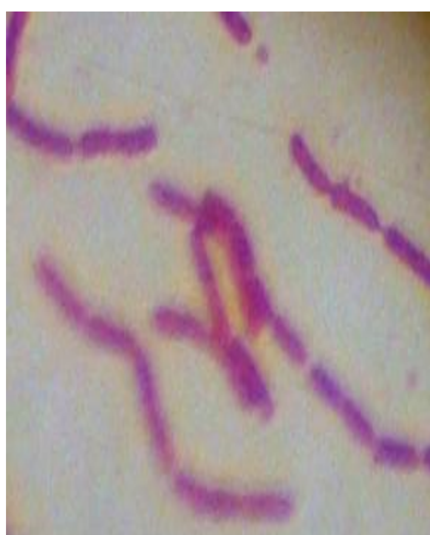
Figure 5. Chromatographic profiles for biodegradation of carbofuran showing the carbofuran peak and its metabolites (1), (2), (3) and (4) at various days of incubation.

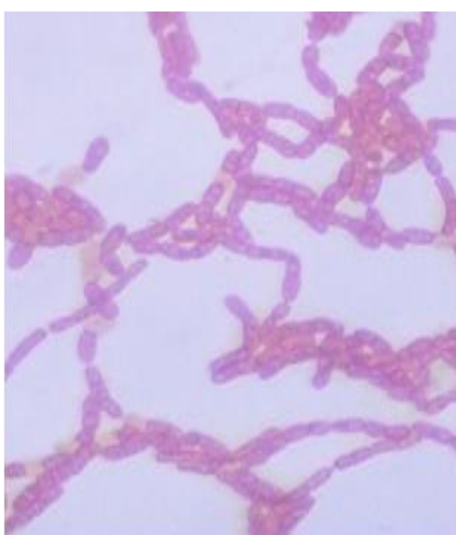
of incubation. Even though this study did not establish the chemical components of the methomyl metabolites, both the methomyl and its metabolites had 100% depletion within 40 days. This suggests that methomyl has a shorter half life in the environment compared to carbofuran whose metabolites could still be detected after 100 days. Methomyl is reported to degrade rapidly in alkaline solution (pH 8.8) and relatively high temperature (WHO, 1996). The initial biodegradation product of methomyl is S-Methyl-N-hydroxythioacetimidate followed by its breakdown to carbon dioxide (WHO, 1996). The high rate of degradation of methomyl may be attributed by different pathways of its degradation including photolysis which yields methomyl oxime as the main metabolite (Tamimi et al., 2005). This experiment was set in the dark to avoid the effect of light on the degradation of methomyl.

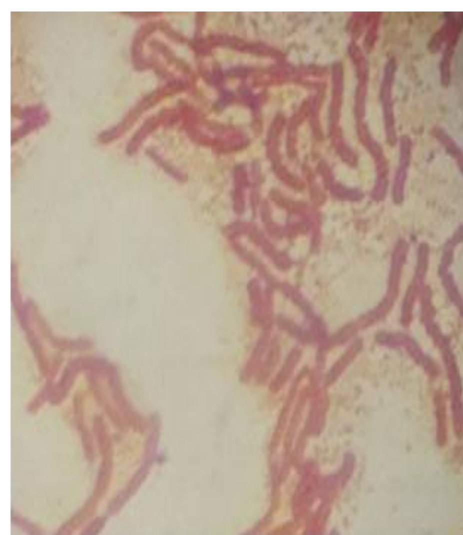
Morphological and biochemical characterization

The isolated strains were gram-negative, rod-shaped (Figure 7). The methomyl degrading strain M-23 and carbofuran degrading strain C-4 were found to possess cytochrome oxidase enzyme thus was able to test positive with N'N'N'N-tetramethyl-p-phenylenediamine dihydrochloride which act as an artificial electron acceptor for the enzyme oxidase. The strains also possessed catalase enzyme which was able to form hydrogen peroxide as an oxidative end product of the aerobic breakdown of sugars. Strains (C-4 and M-23) were able to reduce urea and citrate, growing optimally at a temperature range of 25 to 30°C. Phylogenetic analysis of the strain place them in the same group as *Alcaligenes faecalis* which have been reported to have multidegradative capability and can utilize both methomyl

Figure 6. A chromatographic profile for biodegradation of methomyl showing the methomyl peak and its metabolites (1), (2) and (3). MS is a peak for mineral salt.







Isolate M-14 Isolate C-4 Isolate C-9

Figure 7. Rod shaped bacterial cells of isolates M-14, C-4 and C-9.

Table 1. Summary of morphological and biochemical characteristics of Carbofuran and methomyl degrading strains.

Isolate	C-9	C-4	M-23	M-14
Morphology				
Shape	Rod	Rod	Rod	Rod
Mortility	+	+	+	+
Gram stain	-	-	-	-
Biochemical test				
Gelatinase	+	+	+	+
Nitrate reduction	+	+	+	+
Methyl red	+	+	+	+
Voges-proskeaur	-	+	+	+
Catalase	+	+	+	+
Oxidase	+	+	+	+
Urease	+	+	+	+
Citrate	-	+	+	+
H_2S	-	-	-	+
Indole	-	-	-	+

and carbofuran as observed in this study. Xiaquiang et al. (2005) isolated *Alcaligenes* strain which was able to degrade batch phenol in polyurethane foams. Partial sequence analysis of the amplified 16S gene confirmed that the carbofuran degrading isolate C-9 was closely related to *Pseudomonas pituda*. The strain was rod shaped and gram negative. The strain was negative for indole production test and thus unable to utilize the amino acid tryptophan as a carbon and energy source to produce indole. The isolated strain (C-9) was negative with methyl red and Voges-Proskauer test thus were unable to oxidize glucose with the production and stabilization of high concentration of acids as end products. The isolate had multiple polar flagella for motility and grew optimally at a temperature range of 32 to 35°C. Methomyl degrading isolate M-14 was gram negative, rod shaped and motile. The isolated strain M-14 was able to reduce nitrates to nitrites thus showed red colour in the presence of sulfinic acid and alpha-naphthylamine. The strain was able to oxidize glucose to produce and stabilize high level of acids as end products thus were positive with both methyl red and voges-proskauer tests. The isolate M-14 possess cytochrome oxidase thus was able to catalyse transport of electron from donor compound (NADH) to electron acceptor, in this case, N’N’N’N-tetra methyl-p-phenylenediamine dihydrochloride, indicating indophenol blue colour with oxidase test (Table 1).

Phosphatase and esterase activities

The isolates showed the production of phosphatase and esterase which are key enzymes in biodegradation. The level of phosphatase was estimated by production of p-nitrophenolate and calculated against standard p-nitrophenolate (Figure 1). The levels of phosphatase and esterase were found to be high in carbofuran degrading isolate C-9 compared to isolate C-4. Methomyl degrading isolate M-14 also had significantly higher enzymes production than isolate M-23. This may have been the reason for the ease with which isolate C-9 degraded carbofuran and isolate M-14 degraded methomyl (Figure 8 and Table 2). Although this study did not establish conditions that favour the production of these enzymes, the production of these biodegradative enzymes are reported to be host specific and their activity depends much on growth conditions (Kanekar et al., 2004). However, members of the genera *Pseudomonas* and *Flavobacterium* had been reported to produce hydrolytic enzymes. Phosphotriesterase, a parathione hydrolase, has been cloned from *Flavobactriuum* (Rowland et al., 1991). The difference in the level of both enzymes in the isolates may have contributed to the subsequent differences in the rate of biodegradation.

Molecular characterisation of methomyl and carbofuran degrading strains

The taxonomic classification of the isolated strains was performed using 16S ribosomal DNA sequences of their genomic DNA, which place the carbofuran degrading strains into genera *Pseudomonas* and *Alcaligenes* while the methomyl degrading strains were placed into genera *Flavobacterium* and *Alcaligenes*. Biochemical and morphological characteristics of the strains also support these genus assignments. Phylogenetic analysis of

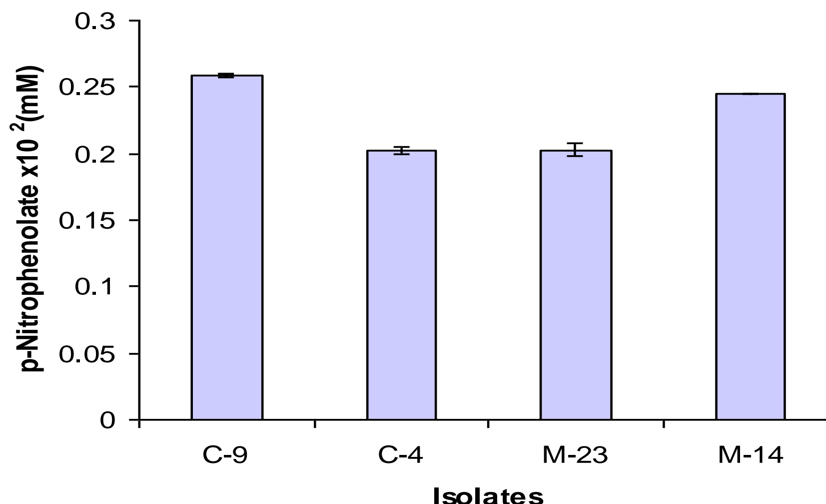


Figure 8. p-Nitrophenolate produced by different isolates measured by sphectrophotometer at 405 nm and calculated against standard p-Nitrophenolate.

Table 2. Esterase activities for different isolates.

Isolate	Colony diameter	Halo diameter	Enzymatic index(IE)
C-9	11.5±1	20.5±0.5	1.78261±0.01422
C-4	11±0.5	6.7±0.5	0.6091±0.0143
M-14	11.5±0.5	6.9±0.5	0.6±0.061
M-23	11±1	6.5±0.5	0.592±0.00833

methomyl degrading strain, M-14 showed that it was closely related to the members of the *Flavobacterium odotarum* with 99% rDNA sequence similarity (Figure 9). This strain was found to efficiently degrade methomyl within 40 days and also degrades carbofuran but at a slower rate (data not shown). The ability of the strain to utilize both methomyl and carbofuran displays the multidegradative capability of the strain. This finding corroborates with other findings where a number of *Flavobacterium* strains have been isolated and reported to degrade short carbon chains to very long carbon chains of petroleum hydrocarbons (Mishra et al., 2001). Some species of *Flavobacterium* have been isolated from sediment and water of a crude oil polluted river and assessed in terms of ability or inability to grow in the presence of 0.5% (v/v) of diesel oil and potential to degrade the diesel oil (Kayode-Isola et al*.,* 2008). *Flavobacterium* has also been used in bioremediation due to its effectiveness in cleaning up petrochemical wastewaters (Shokrollahzadeh et al., 2008). The biodegradability capability of *Flavobacterium* sp. is not limited to oil contaminated environments but has also been found to degrade various pesticides including carbamates as noted in this research and other studies (Seo et al., 2007; Chaudhry and Ali, 1988). Partial sequence analysis of the amplified 16S gene confirmed that the carbofuran degrading isolate C-9 was closely related to *P. pituda* with rDNA sequence analysisis of 99%. The strain C-9 was able to degrade carbofuran efficiently within 100 days at 30°C incubation. This study corroborates with that of Seo et al. (2007) who also found out that, members of the genus *Pseudomonas* strains are able to efficiently utilize carbofuran and its metabolites as a carbon source. *P. putida* utilizes 1 and 2-methylnaphthalene as the sole source of carbon and energy (Mahajan et al., 1994). The complete pathway for carbaryl (1-naphthyl-*N* methylcarbamate) degradation has been elucidated for *Pseudomonas* sp. These strains were found to utilize carbaryl via the 1-naphthol-1, 2-dihydroxynaphthalene, salicylate, gentisate pathway to the central carbon pathway (Swetha and Phale, 2005). *Pseudomonades* have also been found to degrade tri-aromatic compounds such as anthracenes and phenanthrenes (Yemashova et al., 2007). The carbofuran-degrading strains C-4 and methomyl degrading strain M-23 were found to be closely related to members of the genus *Alcaligenes.* The phylogenetic analysis of the 16S rDNA of the two strains showed that they were related to *A. faecalis* with sequence similarity of 99% (Figure 9).

The isolates were able to degrade both carbofuran and methomyl completely confirming the metabolic versatility of *A. faecalis. Alcaligenes* are reported to have wide ecological distribution contributed by their metabolic

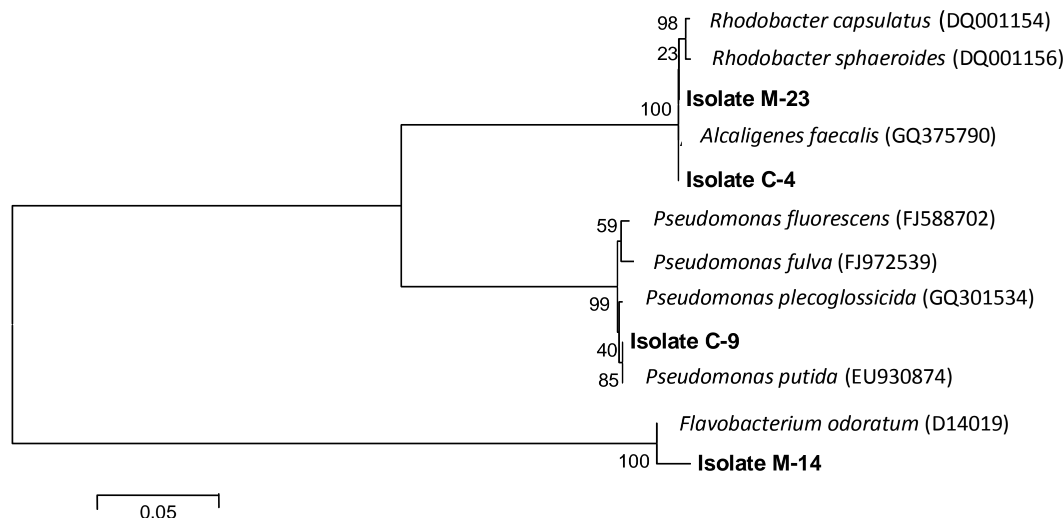


Figure 9. Neighbour-joining phylogenetic tree showing the position of carbofuran and methomyl-degrading isolates M-14, M-23, C-4 and C-9 with reference organisms from the genus *Alcaligenes* and *Pseudomonas.* respectively. The bar indicates the estimated substitution per nucleotide position.

diversity. Jiang et al. (2007) isolated *A. faecalis* from acclimated activated sludge, collected from municipal gas works and it has been shown that immobilized *A. faecalis* are also able to biodegrade phenol. Some members of this group like the *Alcaligenes dinifricans, Alcaligenes odorans,* and *Alcaligenes eutrophus* are known to be excellent hydrocarbon degraders including the polycyclic aromatic hydrocarbons (Harayama et al., 1999).

Apart from metabolic diversity of these pesticide degrading strains, they are also ecologically and geographically distributed as observed in this study and other studies (Song et al., 2000). At least, for each sampling region, a degrading strain was isolated. Owing to the culture dependent method approach used in this study; diverse biodegradative strains could have remained un-isolated despite their presence in the study regions. Advanced molecular techniques that target biodegradative genes and can identify diverse bacterial strains with biodegradation potentials could be used to identify such strains.

CONCLUSIONS AND RECOMMENDATIONS

The isolation of carbofuran- and methomyl-degrading bacteria from horticultural soils in different geographical regions indicates the presence of pesticide degrading bacteria in horticultural soils with a history of pesticide application. This research focused on laboratory culture based techniques which may have limited the diversity of isolated bacteria. We project that more genetically diverse consortium of bacteria with diverse metabolic versatility are likely to be captured if non-culture based techniques were to be employed. From the high pressure liquid chromatography profiles obtained in this study, carbofuran and methomyl can be degraded completely thus may not cause considerable threat to the environment, however, the fate and toxicity of the metabolites especially those of carbofuran should be investigated further. In addition, the knowledge about the biochemical pathways and respective genes involved in biodegradation should be embraced. This understanding should help deal with situations where toxic metabolites are generated to enable safer and sustainable bioremediation strategies. The recent advance in genetic engineering and biotechnology has great potential in exploiting the degradative properties of microorganisms and that the isolates obtained in this study should provide a novel application in environmental biotechnology.

ACKNOWLEDGEMENT

This work was supported by Research Production and Extension (RPE) division, Jomo Kenyatta University of Agriculture and Technology (JKUAT). We also thank institute for Biotechnology research (IBR) for providing laboratory for the research.

REFERENCES

Bartholomew JW (1962). Variables influencing results and precise definition steps in gram staining as a means of standardizing the results obtained. Stain Technol., 37: 139-155.

Bolton PG, Dean ACR (1972). Phosphatase synthesis in *Klebsiella* (*Aerobacter*) *aerogenes* growing in continuous culture. J. Biochem, 127: 87-96.

Cappuccino JG, Sherman N (2002). Microbiology: a laboratory manual (6th Ed) Pearson Education, Inc. San Francisco, CA.
Chaudhry R, Ali AN (1988). Bacterial Metabolism of Carbofurant Appl. Environ. Microbiol., (54)6: 1414-1419.
Chaudhry GR (2002). Induction of carbufuran oxidation to 4-hydroxycarbofuran by Pseudomonas sp.50432. FEMS, Microbiol. Lett., 214(2): 171–176.
Embley TM, Stackebrandt E (1994). The molecular phylogeny and systematic of the actinomycetes. Annual Rev. Microbiol., 48: 257-289.
Gibson J, Harwood CS (2002). Metabolic diversity in aromatic compounds utilization by anaerobic microbes. Annual. Rev. Microbiol. 56: 345-369.
Harayama S, Keshira H, Kasai Y, Shutsubo K (1999). Petroleum biodegradation in marine environments. J. Molecular Microbiol. Biotechnol,. 1: 63-70.
Jiang Y, Wen J, Bai J, Jia X, Hu Z (2007). Biodegradation of phenol at high initial concentration by *Alcaligenes faecalis*. J. Hazard. Mater. 147(1-2): 672-676.
Kanekar P, Bhadbhade B, Deshpande MN, Sarnaik S (2004). Biodegradation of organophosphorus pesticides. Proc. Indian Nat. Sci. Acad., 1: 57-70.
Kayode-Isola TM, Eniola KIT, Olayemi AB, Igunnugbemi OO (2008). Response of Resident Bacteria of a Crude Oil-Polluted River to Diesel Oil. J. Agron., 1: 06-09.
Maarten U, Steven V, Pierre W, Annemie R, Dirk S (2007). Characterization of Cultures Enriched from Acidic Polycyclic Aromatic Hydrocarbon-Contaminated Soil for Growth on Pyrene at Low pHs. Appl. Environ. Microbiol., 73(10): 3159-3164.
Mahajan MC, Phale PS, Vaidyanathan CS (1994). Evidence for the involvement of multiple pathways in the biodegradation of 1- and 2-methylnaphthalene by *Pseudomonas putida* CSV86. Archives Microbiol., 161: 425–433.
Mishra S, Jyot J, Kuhad RC, Lal B (2001). Evaluation of inoculum addition to stimulate *in situ* bioremediation of oily-sludge contaminated soil. Appl. Environ. Microbiol., 67: 1675–1681.
Ngowi AVF, Mbise TJ, Ijani ASM, London L, Ajayi OC (2007). Pesticides use by smallholder farmers in vegetable production in Northern Tanzania. Crop Protect., 26(11): 1617–1624.
Rossberg M, Lendle W, Togel AEL, Dreher LE, Rassaeerts H, Kleinschmid P, Strack H, Beck U, Lipper KA, Trokelson TR, Loser E, Beutel KK (1986). Chlorinated hydrocarbon, In W. Gerhartz (ed.), Ullmann's encyclopedia of industrial chemistry. VCH, Weinheim, Germany, pp. 233-398.
Roux KH (1995). Optimization and troubleshooting in PCR, In Dieffenbach C. W. and G.S. Dveksler (Ed), PCR primer: A laboratory manual. Cold Spring Harbor Laboratory Press, Plainsview, N. Y, pp. 53-62.
Rowland SS, Speedie MK, Pogell BM (1991). Purification and characterization of a secreted recombinant phosphotriesterase from *Streptomyces.* Appl. Environ. Microbiol., 57: 440-444.
Sambrook J, Russel DW (2001). Molecular cloning: a laboratory manual. 3rd ed. Cold Spring Harbor (NY): Cold Spring Harbor Laboratory Press.
Sambrook J, Maniatis T, Fritsch EF (1989). Molecular cloning: A laboratory manual. New York: Cold Spring Habour, Laboratory.
Seo JS, Keum YS, Harada RM, Li QX (2007). Isolation and characterization of bacteria capable of degrading polycyclic aromatic hydrocarbons (PAHs) and organophosphorus pesticides from PAHs-contaminated soil in Hilo, Hawaii. Agric. Food Chem. 55: 5383–5389.
Shayne JJ, Hugenholtz P, Sangwan P, Osborne C, Janssen HP (2003). Laboratory cultivation of widespread and previously uncultured soil bacteria. Appl. Environ. Microbial., 69: 7211-7214.
Shokrollahzadeh S, Azizmohseni F, Golmohammad FH, Shokouhi F (2008). Biodegradation potential and bacterial diversity of a petrochemical waste water treatment plant in Iran. Bioresource Technol. London, 99: 6127-6133.
Sierra G (1957). A simple method for the detection of lipolytic activity of micro- organisms and some observations on the influence of the contact between cells and fatty substrates. Antonie van Leeuwenhoek, 23: 15-22.
Song B, Norberto J, Palleroni NJ, Max MH (2000). Isolation and Characterization of Diverse Halobenzoate-Degrading Denitrifying Bacteria from Soils and Sediments. Appl. Environ. Microbiol. 66(8): 3446–3453.
Swetha VP, Phale PS (2005). The metabolism of carbaryl via 1, 2-dihydroxy naphthalene by soil isolates, Pseudomonas sp. strain C4, C5 and C6. Appl. Environ. Microbiol., 71: 5951-5956.
Tamimi M, Qourzal A, Assabbane J, Choveon M, Ferronato C, Ait-Ichou Y (2005). Photocatalytic degradation of pesticide Methomyl: determination of the reaction pathway and identification of intermediate products. Photochem. Photobiol. Sci., 5: 477-482.
WHO (1986). Carbamate pesticides: A general introduction. Geneva, World Health Organization.
WHO (1996). Environmental health criteria for methomyl; 178. Geneva, World Health Organization.
Xiaquiang J, Jianping W, Yan J, Jing B, Xianrui C (2005). Modeling for Batch Phenol Biodegradation with Immobilized *Alcaligenes faecalis.* AIChE J., 52(4): 1294-1303.
Yemashova A, Natalia VP, Murygina, Dmitry V, Zhukov AA, Zakharyantz MA, Gladchenko VA, Sergey VK (2007). Biodeterioration of crude oil and oil derived products: A review. Rev. Environ. Sci. Biotechnol., 6: 315–337.